L10 ANSWER 1 OF 17 SCISEARCH COPYRIGHT (c) 2008 The Thomson Corporation on STN

ACCESSION NUMBER: 2007:386764 SCISEARCH
THE GENUINE ARTICLE: 147OT
TITLE: A two-base mechanism for Escherichia coli ADP-L-glycero-D-manno-heptose 6-epimerase
AUTHOR: Morrison, James P.; Tanner, Martin E. (Reprint)
CORPORATE SOURCE: Univ British Columbia, Dept Chem, Vancouver, BC V6T 1Z1, Canada (Reprint)
mtanner@chem.ubc.ca
COUNTRY OF AUTHOR: Canada
SOURCE: BIOCHEMISTRY, (27 MAR 2007) Vol. 46, No. 12, pp. 3916-3924.
ISSN: 0006-2960.
PUBLISHER: AMER CHEMICAL SOC, 1155 16TH ST, NW, WASHINGTON, DC 20036 USA.
DOCUMENT TYPE: Article; Journal
LANGUAGE: English
REFERENCE COUNT: 43
ENTRY DATE: Entered STN: 19 Apr 2007
Last Updated on STN: 19 Apr 2007

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB ADP-L-glycero-D-manno-heptose 6-epimerase (HldD or AGME, formerly RfaD) catalyzes the inversion of configuration at C-6' ' of the heptose moiety of ADP-D-glycero-D-manno-heptose and ADP-L-glycero-D-manno-heptose. The epimerase HldD operates in the biosynthetic pathway of L-glycero-D-manno-heptose, which is a conserved sugar in the core region of lipopolysaccharide (LPS) of Gram-negative bacteria. Previous studies support a mechanism in which HldD uses its tightly bound NADP(+) cofactor to oxidize directly at C-6' ', generating a ketone intermediate. A reduction of the ketone from the opposite face then occurs, generating the epimeric product. How the epimerase is able access both faces of the ketone intermediate with correct alignment of the three required components, NADPH, the ketone carbonyl, and a catalytic acid/base residue, is addressed here. It is proposed that the epimerase active site contains two catalytic pockets, each of which bears a catalytic acid/base residue that facilitates reduction of the C-6' ' ketone but leads to a distinct epimeric product. The ketone carbonyl may access either pocket via rotation about the C-5' '-C-6' ' bond of the sugar nucleotide and in doing so presents opposing faces to the bound cofactor. Evidence in support of the two-base mechanism is found in studies of two single mutants of the Escherichia coli K-12 epimerase, Y140F and K178M, both of which have severely compromised epimerase activities that are more than 3 orders of magnitude lower than that of the wild type. The catalytic competency of these two mutants in promoting redox chemistry is demonstrated with an alternate catalytic activity that requires only one catalytic base: dismutation of a C-6' ' aldehyde substrate analogue (ADP-beta-D-manno-hexodialdose) to an acid and an alcohol (ADP-beta-D-mannuronic acid and ADP-beta-D-mannose). This study identifies the two catalytic bases as tyrosine 140 and lysine 178. A one-step enzymatic conversion of mannose into ADP-beta-mannose is also described and used to make C-6' '-substituted derivatives of this sugar nucleotide.

L10 ANSWER 2 OF 17 SCISEARCH COPYRIGHT (c) 2008 The Thomson Corporation on STN

ACCESSION NUMBER: 2008:9802 SCISEARCH
THE GENUINE ARTICLE: 237VF
TITLE: Predicting protein function from structure - The roles of

short-chain dehydrogenase/reductase enzymes in Bordetella O-antigen biosynthesis
AUTHOR: King, Jerry D. (Reprint); Harmer, Nicholas J.; Preston, Andrew; Palmer, Colin M.; Rejzek, Martin; Field, Robert A.; Blundell, Tom L.; Maskell, Duncan J.
CORPORATE SOURCE: Univ Guelph, Dept Mol & Cellular Biol, Guelph, ON N1G 2W1, Canada (Reprint); Univ Cambridge, Dept Vet Med, Cambridge CB3 0ES, England; Univ Cambridge, Dept Biochem, Cambridge CB2 1GA, England; Univ E Anglia, Sch Chem Sci & Pharm, Norwich NR4 7TJ, Norfolk, England
jking01@uoguelph.ca
COUNTRY OF AUTHOR: Canada; England
SOURCE: JOURNAL OF MOLECULAR BIOLOGY, (30 NOV 2007) Vol. 374, No. 3, pp. 749-763.
ISSN: 0022-2836.
PUBLISHER: ACADEMIC PRESS LTD ELSEVIER SCIENCE LTD, 24-28 OVAL RD, LONDON NW1 7DX, ENGLAND.
DOCUMENT TYPE: Article; Journal
LANGUAGE: English
REFERENCE COUNT: 69
ENTRY DATE: Entered STN: 3 Jan 2008
Last Updated on STN: 3 Jan 2008
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB The pathogenic bacteria Bordetella parapertussis and Bordetella bronchiseptica express a lipopolysaccharide O antigen containing a polymer of 2,3-diacetamido-2,3-dideoxy-L-galacturonic acid. The O-antigen cluster contains three neighbouring genes that encode proteins belonging to the short-chain dehydrogenase/reductase (SDR) family, wbmF, wbmG and wbmH, and we aimed to elucidate their individual functions. Mutation and complementation implicate each gene in C-antigen expression but, as their putative sugar nucleotide substrates are not currently available, biochemical characterisation of WbmF, WbmG and WbmH is impractical at the present time. SDR family members catalyse a wide range of chemical reactions including oxidation, reduction and epimerisation. Because they typically share low sequence conservation, however, catalytic function cannot be predicted from sequence analysis alone. In this context, structural characterisation of the native proteins, co-crystals and small-molecule soaks enables differentiation of the functions of WbmF, WbmG and WbmH. These proteins exhibit typical SDR architecture and coordinate NAD. In the substrate-binding domain, all three enzymes bind uridyl nucleotides. WbmG contains a typical SDR catalytic TYK triad, which is required for oxidoreductase function, but the active site is devoid of additional acid-base functionality. Similarly, WbmH possesses a TYK triad, but an otherwise feature-poor active site. Consequently, 3,5-epimerase function can probably be ruled out for these enzymes. The WbmF active site contains conserved 3,5-epimerase features, namely, a positionally conserved cysteine (Cys133) and basic side chain (His90 or Asn213), but lacks the serine/ threonine component of the SDR triad and therefore may not act as an oxidoreductase. The data suggest a pathway for synthesis of the O-antigen precursor UDP-2,3-diacetamido-2,3-dideoxy-L-galacturonic acid and illustrate the usefulness of structural data in predicting protein function. (c) 2007 Elsevier Ltd. All rights reserved.

L10 ANSWER 3 OF 17 MEDLINE on STN DUPLICATE 1
ACCESSION NUMBER: 2007603829 IN-PROCESS
DOCUMENT NUMBER: PubMed ID: 17762900
TITLE: High-resolution structure of NodZ fucosyltransferase involved in the biosynthesis of the nodulation factor.
AUTHOR: Brzezinski Krzysztof; Stepkowski Tomasz; Panjikar Santosh;

Bujacz Grzegorz; Jaskolski Mariusz
CORPORATE SOURCE: Department of Crystallography, Faculty of Chemistry, A. Mickiewicz University, Poznan, Poland.
SOURCE: Acta biochimica Polonica, (2007) Vol. 54, No. 3, pp. 537-49. Electronic Publication: 2007-08-30.
Journal code: 14520300R. ISSN: 0001-527X.
PUB. COUNTRY: Poland
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: NONMEDLINE; IN-DATA-REVIEW; IN-PROCESS; NONINDEXED; Priority Journals
ENTRY DATE: Entered STN: 12 Oct 2007
Last Updated on STN: 10 Dec 2007

AB The fucosyltransferase NodZ is involved in the biosynthesis of the nodulation factor in nitrogen-fixing symbiotic bacteria. It catalyzes alpha1,6 transfer of l-fucose from GDP-fucose to the reducing residue of the synthesized Nod oligosaccharide. We present the structure of the NodZ protein from Bradyrhizobium expressed in Escherichia coli and crystallized in the presence of phosphate ions in two crystal forms. The enzyme is arranged into two domains of nearly equal size. Although NodZ falls in one broad class (GT-B) with other two-domain glycosyltransferases, the topology of its domains deviates from the canonical Rossmann fold, with particularly high distortions in the N-terminal domain. Mutational data combined with structural and sequence alignments indicate residues of potential importance in GDP-fucose binding or in the catalytic mechanism. They are all clustered in three conserved sequence motifs located in the C-terminal domain.

L10 ANSWER 4 OF 17 SCISEARCH COPYRIGHT (c) 2008 The Thomson Corporation on STN

ACCESSION NUMBER: 2007:85349 SCISEARCH
THE GENUINE ARTICLE: 122AY
TITLE: RmlC, a C3 ' and C5 ' carbohydrate epimerase, appears to operate via an intermediate with an unusual twist boat conformation
AUTHOR: Dong, Changjiang; Major, Louise L.; Srikannathasan, Velupillai; Errey, James C.; Giraud, Marie-France; Lam, Joseph S.; Graninger, Michael; Messner, Paul; McNeil, Michael R.; Field, Robert A.; Whitfield, Chris; Naismith, James H. (Reprint)
CORPORATE SOURCE: Univ St Andrews, Ctr Biomol Sci, St Andrews KY16 9ST, Fife, Scotland (Reprint); Univ E Anglia, Sch Chem Sci & Pharm, Norwich NR4 7TJ, Norfolk, England; Univ Guelph, Dept Mol & Cellular Biol, Guelph, ON N1G 2W1, Canada; Agr Univ Vienna, Zentrum NanoBiotechnol, A-1180 Vienna, Austria; Colorado State Univ, Dept Microbiol, Ft Collins, CO 80523 USA
naismith@st-and.ac.uk
COUNTRY OF AUTHOR: Scotland; England; Canada; Austria; USA
SOURCE: JOURNAL OF MOLECULAR BIOLOGY, (5 JAN 2007) Vol. 365, No. 1, pp. 146-159.
ISSN: 0022-2836.
PUBLISHER: ACADEMIC PRESS LTD ELSEVIER SCIENCE LTD, 24-28 OVAL RD, LONDON NW1 7DX, ENGLAND.
DOCUMENT TYPE: Article; Journal
LANGUAGE: English
REFERENCE COUNT: 51
ENTRY DATE: Entered STN: 25 Jan 2007

Last Updated on STN: 25 Jan 2007
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB The striking feature of carbohydrates is their constitutional, conformational and configurational diversity. Biology has harnessed this diversity and manipulates carbohydrate residues in a variety of ways, one of which is epimerization. Rml C catalyzes the epimerization of the C3' and C5' positions of dTDP-6-deoxy-D-xylo-4-hexulose, forming dTDP-6-deoxy-L-lyxo-4-hexulose. Rml C is the third enzyme of the rhamnose pathway, and represents a validated anti-bacterial drug target. Although several structures of the enzyme have been reported, the mechanism and the nature of the intermediates have remained obscure. Despite its relatively small size (22 kDa), Rml C catalyzes four stereospecific proton transfers and the substrate undergoes a major conformational change during the course of the transformation. Here we report the structure of Rml C from several organisms in complex with product and product mimics. We have probed site-directed mutants by assay and by deuterium exchange. The combination of structural and biochemical data has allowed us to assign key residues and identify the conformation of the carbohydrate during turnover. Clear knowledge of the chemical structure of Rml C reaction intermediates may offer new opportunities for rational drug design.

L10 ANSWER 5 OF 17 SCISEARCH COPYRIGHT (c) 2008 The Thomson Corporation on STN
ACCESSION NUMBER: 2001:584769 SCISEARCH
THE GENUINE ARTICLE: 454NP
TITLE: The total synthesis of lipid I
AUTHOR: VanNieuwenhze M S (Reprint); Mauldin S C; Zia-Ebrahimi M; Aikins J A; Blaszczak L C
CORPORATE SOURCE: Eli Lilly & Co, Lilly Corp Ctr, Discovery Chem Res & Chem Proc Res & Dev, Lilly Res Labs, Indianapolis, IN 46285 USA (Reprint)
COUNTRY OF AUTHOR: USA
SOURCE: JOURNAL OF THE AMERICAN CHEMICAL SOCIETY, (25 JUL 2001) Vol. 123, No. 29, pp. 6983-6988. ISSN: 0002-7863.
PUBLISHER: AMER CHEMICAL SOC, 1155 16TH ST, NW, WASHINGTON, DC 20036 USA.
DOCUMENT TYPE: Article; Journal
LANGUAGE: English
REFERENCE COUNT: 55
ENTRY DATE: Entered STN: 3 Aug 2001
Last Updated on STN: 3 Aug 2001
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB A total synthesis of lipid I (4), a membrane-associated intermediate in the bacterial cell wall (peptidoglycan) biosynthesis pathway, is reported. This highly convergent synthesis will enable further studies on bacterial resistance mechanisms and may provide insight toward the development of new chemotherapeutic agents with novel modes of action.

L10 ANSWER 6 OF 17 SCISEARCH COPYRIGHT (c) 2008 The Thomson Corporation on STN
ACCESSION NUMBER: 2001:922071 SCISEARCH
THE GENUINE ARTICLE: 490UB
TITLE: Kinetic and crystallographic analyses support a sequential-ordered BiBi catalytic mechanism for Escherichia coli glucose-1-phosphate thymidylyltransferase
AUTHOR: Zuccotti S; Zanardi D; Rosano C; Sturla L; Tonetti M; Bolognesi M (Reprint)

CORPORATE SOURCE: Univ Genoa, INFM, Dept Phys, Via Dodecaneso 33, I-16146 Genoa, Italy (Reprint); Univ Genoa, INFM, Dept Phys, I-16146 Genoa, Italy; Univ Genoa, Adv Biotechnol Ctr, I-16146 Genoa, Italy; Giannina Gaslini Inst, I-16148 Genoa, Italy; Univ Genoa, Dept Expt Med, Biochem Sect, I-16132 Genoa, Italy; Natl Inst Canc Res, Adv Biotechnol Ctr, I-16132 Genoa, Italy
COUNTRY OF AUTHOR: Italy
SOURCE: JOURNAL OF MOLECULAR BIOLOGY, (2 NOV 2001) Vol. 313, No. 4, pp. 831-843.
ISSN: 0022-2836.
PUBLISHER: ACADEMIC PRESS LTD, 24-28 OVAL RD, LONDON NW1 7DX, ENGLAND
.
DOCUMENT TYPE: Article; Journal
LANGUAGE: English
REFERENCE COUNT: 46
ENTRY DATE: Entered STN: 30 Nov 2001
Last Updated on STN: 30 Nov 2001
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Glucose-1-phosphate thymidylyltransferase is the first enzyme in the biosynthesis of dTDP-L-rhamnose, the precursor Of L-rhamnose, an essential component of surface antigens, such as the O-lipopolysaccharide, mediating virulence and adhesion to host tissues in many microorganisms. The enzyme catalyses the formation of dTDP-glucose, from dTTP and glucose 1-phosphate, as well as its pyrophosphorolysis. To shed more light on the catalytic properties of glucose-1-phosphate thymidylyltransferase from Escherichia coli, specifically distinguishing between ping pong and sequential ordered bi bi reaction mechanisms, the enzyme kinetic properties have been analysed in the presence of different substrates and inhibitors. Moreover, three different complexes of glucose-1-phosphate thymidylyltransferase (co-crystallized with dTDP, with dTMP and glucose-1-phosphate, with D-thymidine and glucose-1-phosphate) have been analysed by X-ray crystallography, in the 1.9-2.3 Angstrom resolution range (R-factors, of 17.3-17.5%). The homotetrameric enzyme shows strongly conserved substrate/inhibitor binding modes in a surface cavity next to the topological switch-point of a quasi-Rossmann fold. Inspection of the subunit tertiary structure reveals relationships to other enzymes involved in the biosynthesis of nucleotide-sugars, including distant proteins such as the molybdenum cofactor biosynthesis protein MobA. The precise location of the substrate relative to putative reactive residues in the catalytic center suggests that, in keeping with the results of the kinetic measurements, both catalysed reactions, i.e. dTDP-glucose biosynthesis and pyrophosphorolysis, follow a sequential ordered bi bi catalytic mechanism. (C) 2001 Academic Press.

L10 ANSWER 7 OF 17 CAPLUS COPYRIGHT 2008 ACS on STN DUPLICATE 2
ACCESSION NUMBER: 2000:133880 CAPLUS
DOCUMENT NUMBER: 132:162817
TITLE: Crystal structure of Escherichia coli GDP-fucose synthetase and methods of its use for identifying the enzyme inhibitors
INVENTOR(S): Somers, William S.; Stahl, Mark L.; Sullivan, Francis X.
PATENT ASSIGNEE(S): American Home Products Corporation, USA
SOURCE: PCT Int. Appl., 56 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English

FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2000009744	A1	20000224	WO 1999-US18441	19990813
W: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, HR, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, UZ, VN, YU, ZW				
RW: GH, GM, KE, LS, MW, SD, SL, SZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG				
CA 2340074	A1	20000224	CA 1999-2340074	19990813
AU 9954836	A	20000306	AU 1999-54836	19990813
EP 1105523	A1	20010613	EP 1999-941121	19990813
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO				
US 6459996	B1	20021001	US 1999-373432	19990813
US 2007038380	A1	20070215	US 2002-90879	20020304
PRIORITY APPLN. INFO.:			US 1998-96452P	P 19980813
			US 1999-373432	A3 19990813
			WO 1999-US18441	W 19990813

AB The present invention provides for crystalline GDP-fucose synthetase (GFS) of E. coli. The crystal structure of GFS and GFS complexes with NADPH and NADP+ has also been solved. Models based upon such crystal structure are also provided. Methods of identifying inhibitors of GFS activity using such models are also disclosed.

REFERENCE COUNT: 7 THERE ARE 7 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L10 ANSWER 8 OF 17 SCISEARCH COPYRIGHT (c) 2008 The Thomson Corporation on STN

ACCESSION NUMBER: 2000:812835 SCISEARCH
THE GENUINE ARTICLE: 367GU
TITLE: The high resolution structure of GDP-4 -keto-6-deoxy-D- mannose epimerase/reductase
AUTHOR: Rosano C; Izzo G; Sturla L; Bisso A; Tonetti M; Bolognesi M (Reprint)
CORPORATE SOURCE: Univ Genoa, INFM, Dept Phys, Largo Rosanna Benzi 10, I-16132 Genoa, Italy (Reprint); Univ Genoa, INFM, Dept Phys, I-16132 Genoa, Italy; Univ Genoa, IST, Adv Biotechnol Ctr, I-16132 Genoa, Italy; Univ Genoa, Dept Expt Med, Biochem Sect, I-16132 Genoa, Italy
COUNTRY OF AUTHOR: Italy
SOURCE: CROATICA CHEMICA ACTA, (SEP 2000) Vol. 73, No. 3, pp. 887-899.
ISSN: 0011-1643.
PUBLISHER: CROATIAN CHEMICAL SOC, MARULICEV TRG 19/II, 41001 ZAGREB, CROATIA.
DOCUMENT TYPE: Article; Journal
LANGUAGE: English
REFERENCE COUNT: 16
ENTRY DATE: Entered STN: 2000
Last Updated on STN: 2000
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB GDP-4-keto-6-deoxy-D-mannose epimerase/reductase is a bifunctional enzyme involved in the biosynthesis of cell-surface structures, such as blood group antigens. Each subunit in the homodimeric enzyme consists of two domains. The N-terminal domain displays a Rossmann-fold topology and binds the NADP(+) coenzyme. The C-terminal domain is held to bind the substrate. The hole-enzyme structure has been refined at 1.45 Angstrom resolution, based on synchrotron data, to a final R-factor of 0.127 (R-free = 0.167). The refined protein model highlights several residues involved in coenzyme recognition and binding and suggests that the enzyme belongs to the short-chain dehydrogenase protein homology family. Implications of the catalytic mechanism are discussed.

L10 ANSWER 9 OF 17 SCISEARCH COPYRIGHT (c) 2008 The Thomson Corporation on STN
ACCESSION NUMBER: 2001:99276 SCISEARCH
THE GENUINE ARTICLE: 394DQ
TITLE: The rhamnose pathway
AUTHOR: Giraud M F (Reprint); Naismith J H
CORPORATE SOURCE: Univ Bordeaux 2, CNRS, Inst Biochim & Genet Cellulaires, 1 Rue Camille St Saens, F-33077 Bordeaux, France (Reprint); Univ Bordeaux 2, CNRS, Inst Biochim & Genet Cellulaires, F-33077 Bordeaux, France; Univ St Andrews, Ctr Biomol Sci, St Andrews KY16 9ST, Fife, Scotland
COUNTRY OF AUTHOR: France; Scotland
SOURCE: CURRENT OPINION IN STRUCTURAL BIOLOGY, (DEC 2000) Vol. 10, No. 6, pp. 687-696.
ISSN: 0959-440X.
PUBLISHER: CURRENT BIOLOGY LTD, 84 THEOBALDS RD, LONDON WC1X 8RR, ENGLAND.
DOCUMENT TYPE: Article; Journal
LANGUAGE: English
REFERENCE COUNT: 47
ENTRY DATE: Entered STN: 9 Feb 2001
Last Updated on STN: 9 Feb 2001
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB L-Rhamnose is a deoxy sugar found widely in bacteria and plants. Evidence continues to emerge about its essential role in many pathogenic bacteria. The crystal structures of two of the four enzymes involved in its biosynthetic pathway have been reported and the other two have been submitted for publication. This pathway does not exist in humans, making enzymes of this pathway very attractive targets for therapeutic intervention.

L10 ANSWER 10 OF 17 MEDLINE on STN DUPLICATE 3
ACCESSION NUMBER: 2000139699 MEDLINE
DOCUMENT NUMBER: PubMed ID: 10673432
TITLE: Structural and kinetic analysis of Escherichia coli GDP-mannose 4,6 dehydratase provides insights into the enzyme's catalytic mechanism and regulation by GDP -fucose.
AUTHOR: Somoza J R; Menon S; Schmidt H; Joseph-McCarthy D; Dessen A; Stahl M L; Somers W S; Sullivan F X
CORPORATE SOURCE: Wyeth Research, Cambridge, MA 02140, USA.
SOURCE: Structure (London, England : 1993), (2000 Feb 15) Vol. 8, No. 2, pp. 123-35.
Journal code: 101087697. ISSN: 0969-2126.
PUB. COUNTRY: ENGLAND: United Kingdom

DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200003
ENTRY DATE: Entered STN: 20 Mar 2000
Last Updated on STN: 30 Oct 2002
Entered Medline: 9 Mar 2000

AB Background: GDP-mannose 4,6 dehydratase (GMD) catalyzes the conversion of GDP-(D)-mannose to GDP-4-keto, 6-deoxy-(D)-mannose. This is the first and regulatory step in the de novo biosynthesis of GDP-(L)-fucose. Fucose forms part of a number of glycoconjugates, including the ABO blood groups and the selectin ligand sialyl Lewis X. Defects in GDP-fucose metabolism have been linked to leukocyte adhesion deficiency type II (LADII). Results: The structure of the GDP-mannose 4,6 dehydratase apo enzyme has been determined and refined using data to 2.3 A resolution. GMD is a homodimeric protein with each monomer composed of two domains. The larger N-terminal domain binds the NADP(H) cofactor in a classical Rossmann fold and the C-terminal domain harbors the sugar-nucleotide binding site. We have determined the GMD dissociation constants for NADP, NADPH and GDP-mannose. Each GMD monomer binds one cofactor and one substrate molecule, suggesting that both subunits are catalytically competent. GDP-fucose acts as a competitive inhibitor, suggesting that it binds to the same site as GDP-mannose, providing a mechanism for the feedback inhibition of fucose biosynthesis. Conclusions: The X-ray structure of GMD reveals that it is a member of the short-chain dehydrogenase/reductase (SDR) family of proteins. We have modeled the binding of NADP and GDP-mannose to the enzyme and mutated four of the active-site residues to determine their function. The combined modeling and mutagenesis data suggests that at position 133 threonine substitutes serine as part of the serine-tyrosine-lysine catalytic triad common to the SDR family and Glu 135 functions as an active-site base.

L10 ANSWER 11 OF 17 MEDLINE on STN DUPLICATE 4
ACCESSION NUMBER: 2001014332 MEDLINE
DOCUMENT NUMBER: PubMed ID: 11021971
TITLE: Probing the catalytic mechanism of GDP-4-keto-6-deoxy-d-mannose Epimerase/Reductase by kinetic and crystallographic characterization of site-specific mutants.
AUTHOR: Rosano C; Bisso A; Izzo G; Tonetti M; Sturla L; De Flora A; Bolognesi M
CORPORATE SOURCE: Department of Physics-INFM and Advanced Biotechnology Center-IST, University of Genova, Largo Rosanna Benzi 10, Genova, I-16132, Italy.
SOURCE: Journal of molecular biology, (2000 Oct 13) Vol. 303, No. 1, pp. 77-91.
Journal code: 2985088R. ISSN: 0022-2836.
PUB. COUNTRY: ENGLAND: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
(RESEARCH SUPPORT, NON-U.S. GOV'T)
LANGUAGE: English
FILE SEGMENT: Priority Journals
OTHER SOURCE: PDB-1E6U; PDB-1E7Q; PDB-1E7R; PDB-1E7S
ENTRY MONTH: 200010
ENTRY DATE: Entered STN: 22 Mar 2001
Last Updated on STN: 22 Mar 2001
Entered Medline: 31 Oct 2000

AB GDP-4-keto-6-deoxy-
d-mannose epimerase/reductase is a
bifunctional enzyme responsible for the last step in the biosynthesis of
GDP-l-fucose, the substrate of fucosyl transferases. Several cell-surface
antigens, including the leukocyte Lewis system and cell-surface antigens
in pathogenic bacteria, depend on the availability of GDP-l-fucose for
their expression. Therefore, the enzyme is a potential target for therapy
in pathological states depending on selectin-mediated cell-to-cell
interactions. Previous crystallographic investigations have
shown that GDP-4-keto-6-
deoxy-d-mannose epimerase/
reductase belongs to the short-chain dehydrogenase/reductase
protein homology family. The enzyme active-site region is at the
interface of an N-terminal NADPH-binding domain and a C-terminal domain,
held to bind the substrate. The design, expression and functional
characterization of seven site-specific mutant forms of GDP-
4-keto-6-deoxy-d-
mannose epimerase/reductase are reported here.
In parallel, the crystal structures of the native holoenzyme and
of three mutants (Ser107Ala, Tyr136Glu and Lys140Arg) have been
investigated and refined at 1. 45-1.60 A resolution, based on synchrotron
data (R-factors range between 12.6 % and 13.9 %). The refined protein
models show that besides the active-site residues Ser107, Tyr136 and
Lys140, whose mutations impair the overall enzymatic activity and may
affect the coenzyme binding mode, side-chains capable of proton exchange,
located around the expected substrate (GDP-4-keto-6-deoxy-d-mannose)
binding pocket, are selectively required during the epimerization and
reduction steps. Among these, Cys109 and His179 may play a primary role
in proton exchange between the enzyme and the epimerization catalytic
intermediates. Finally, the additional role of mutated active-site
residues involved in substrate recognition and in enzyme stability has
been analyzed.

L10 ANSWER 12 OF 17 SCISEARCH COPYRIGHT (c) 2008 The Thomson Corporation on STN

ACCESSION NUMBER:	2000:595682 SCISEARCH
THE GENUINE ARTICLE:	340LB
TITLE:	Site-directed mutagenesis and crystallographic studies of GDP-4-keto-6-deoxy-D-mannose epimerase/reductase (GMER) from E-coli
AUTHOR:	Tonetti M (Reprint); Rosano C; Bisso A; Izzo G; Zuccotti S; De Flora A; Bolognesi M
CORPORATE SOURCE:	Univ Genoa, Dept Expt Med, Biochem Sect, Lgo Rosanna Benzi 10, I-16132 Genoa, Italy (Reprint); Univ Genoa, Dept Expt Med, Biochem Sect, I-16132 Genoa, Italy; IST, CBA, Adv Biotechnol Ctr, I-16132 Genoa, Italy; Dept Phys, I-16132 Genoa, Italy; Univ Genoa, INFM, I-16132 Genoa, Italy
COUNTRY OF AUTHOR:	Italy
SOURCE:	ITALIAN JOURNAL OF BIOCHEMISTRY, (MAR 2000) Vol. 49, No. 1, pp. 12-12. ISSN: 0021-2938.
PUBLISHER:	BIOMEDIA SRL, VIA CARLO FARINI 70, 20159 MILAN, ITALY.
DOCUMENT TYPE:	Article; Journal
LANGUAGE:	English
REFERENCE COUNT:	5
ENTRY DATE:	Entered STN: 2000

Last Updated on STN: 2000

L10 ANSWER 13 OF 17 MEDLINE on STN DUPLICATE 5
ACCESSION NUMBER: 1999410404 MEDLINE
DOCUMENT NUMBER: PubMed ID: 10480878
TITLE: Stereochemical course and steady state mechanism of the reaction catalyzed by the GDP-fucose synthetase from Escherichia coli.
AUTHOR: Menon S; Stahl M; Kumar R; Xu G Y; Sullivan F
CORPORATE SOURCE: Wyeth Research, Cambridge, Massachusetts 02140, USA.. smenon@genetics.com
SOURCE: The Journal of biological chemistry, (1999 Sep 17) Vol. 274, No. 38, pp. 26743-50. Journal code: 2985121R. ISSN: 0021-9258.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199910
ENTRY DATE: Entered STN: 26 Oct 1999
Last Updated on STN: 26 Oct 1999
Entered Medline: 13 Oct 1999

AB Recently the genes encoding the human and Escherichia coli GDP-mannose dehydratase and GDP-fucose synthetase (GFS) protein have been cloned and it has been shown that these two proteins alone are sufficient to convert GDP mannose to GDP fucose in vitro. GDP-fucose synthetase from E. coli is a novel dual function enzyme in that it catalyzes epimerizations and a reduction reaction at the same active site. This aspect separates fucose biosynthesis from that of other deoxy and dideoxy sugars in which the epimerase and reductase activities are present on separate enzymes encoded by separate genes. By NMR spectroscopy we have shown that GFS catalyzes the stereospecific hydride transfer of the ProS hydrogen from NADPH to carbon 4 of the mannose sugar. This is consistent with the stereospecificity observed for other members of the short chain dehydrogenase reductase family of enzymes of which GFS is a member. Additionally the enzyme is able to catalyze the epimerization reaction in the absence of NADP or NADPH. The kinetic mechanism of GFS as determined by product inhibition and fluorescence binding studies is consistent with a random mechanism. The dissociation constants determined from fluorescence studies indicate that the enzyme displays a 40-fold stronger affinity for the substrate NADPH as compared with the product NADP and utilizes NADPH preferentially as compared with NADH. This study on GFS, a unique member of the short chain dehydrogenase reductase family, coupled with that of its recently published crystal structure should aid in the development of antimicrobial or anti-inflammatory compounds that act by blocking selectin-mediated cell adhesion.

L10 ANSWER 14 OF 17 MEDLINE on STN DUPLICATE 6
ACCESSION NUMBER: 1998192611 MEDLINE
DOCUMENT NUMBER: PubMed ID: 9525924
TITLE: Molecular cloning of human GDP-mannose 4,6-dehydratase and reconstitution of GDP-fucose biosynthesis in vitro.
AUTHOR: Sullivan F X; Kumar R; Kriz R; Stahl M; Xu G Y; Rouse J; Chang X J; Boodhoo A; Potvin B; Cumming D A
CORPORATE SOURCE: Small Molecule Drug Discovery, Genetics Institute, Inc., 424 Wilkinway, Edmonton, Alberta T6M 2H8, Canada.. fsullivan@genetics.com

SOURCE: The Journal of biological chemistry, (1998 Apr 3) Vol. 273, No. 14, pp. 8193-202.
Journal code: 2985121R. ISSN: 0021-9258.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
OTHER SOURCE: GENBANK-AF042377
ENTRY MONTH: 199805
ENTRY DATE: Entered STN: 14 May 1998
Last Updated on STN: 14 May 1998
Entered Medline: 7 May 1998

AB We have cloned the cDNA encoding human GDP-mannose 4,6-dehydratase, the first enzyme in the pathway converting GDP-mannose to GDP-fucose. The message is expressed in all tissues and cell lines examined, and the cDNA complements Lec13, a Chinese Hamster Ovary cell line deficient in GDP-mannose 4,6-dehydratase activity. The human GDP-mannose 4,6-dehydratase polypeptide shares 61% identity with the enzyme from Escherichia coli, suggesting broad evolutionary conservation. Purified recombinant enzyme utilizes NADP+ as a cofactor and, like its E. coli counterpart, is inhibited by GDP-fucose, suggesting that this aspect of regulation is also conserved. We have isolated the product of the dehydratase reaction, GDP-4-keto-6-deoxymannose, and confirmed its structure by electrospray ionization-mass spectrometry and high field NMR. Using purified recombinant human GDP-mannose 4,6-dehydratase and FX protein (GDP-keto-6-deoxymannose 3,5-epimerase, 4-reductase), we show that the two proteins alone are sufficient to convert GDP-mannose to GDP-fucose in vitro. This unequivocally demonstrates that the epimerase and reductase activities are on a single polypeptide. Finally, we show that the two homologous enzymes from E. coli are sufficient to carry out the same enzymatic pathway in bacteria.

L10 ANSWER 15 OF 17 MEDLINE on STN DUPLICATE 7

ACCESSION NUMBER: 1999081889 MEDLINE
DOCUMENT NUMBER: PubMed ID: 9862812
TITLE: GDP-fucose synthetase from Escherichia coli: structure of a unique member of the short-chain dehydrogenase/reductase family that catalyzes two distinct reactions at the same active site.
AUTHOR: Somers W S; Stahl M L; Sullivan F X
CORPORATE SOURCE: Small Molecule Drug Discovery Genetics Institute, Inc. 87 Cambridgepark Drive, Cambridge, MA 02140, USA.
SOURCE: Structure (London, England : 1993), (1998 Dec 15) Vol. 6, No. 12, pp. 1601-12.
Journal code: 101087697. ISSN: 0969-2126.
PUB. COUNTRY: ENGLAND: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
OTHER SOURCE: PDB-1BSV; PDB-1FXS; PDB-1GFS
ENTRY MONTH: 199902
ENTRY DATE: Entered STN: 16 Feb 1999
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AB Background:. In all species examined, GDP-fucose is synthesized from GDP-mannose in a three-step reaction catalyzed by two enzymes, GDP-mannose 4,6 dehydratase and a dual function 3,

5-epimerase-4-reductase named GDP-fucose synthetase. In this latter aspect fucose biosynthesis differs from that of other deoxy and dideoxy sugars, in which the epimerase and reductase activities are present as separate enzymes. Defects in GDP-fucose biosynthesis have been shown to affect nodulation in bacteria, stem development in plants, and are associated with the immune defect leukocyte adhesion deficiency type II in humans. Results:. We have determined the structure of GDP-fucose synthetase from Escherichia coli at 2.2 A resolution. The structure of GDP-fucose synthetase is closely related to that of UDP-galactose 4-epimerase and more distantly to other members of the short-chain dehydrogenase/reductase family. We have also determined the structures of the binary complexes of GDP-fucose synthetase with its substrate NADPH and its product NADP+. The nicotinamide cofactors bind in the syn and anti conformations, respectively. Conclusions:. GDP-fucose synthetase binds its substrate, NADPH, in the proper orientation (syn) for transferring the 4-pro-S hydride of the nicotinamide. We have observed a single binding site in GDP-fucose synthetase for the second substrate, GDP-4-keto,6-deoxy-mannose. This implies that both the epimerization and reduction reactions occur at the same site in the enzyme. As is the case for all members of the short-chain family of dehydrogenase/reductases, GDP-fucose synthetase retains the Ser-Tyr-Lys catalytic triad. We propose that this catalytic triad functions in a mechanistically equivalent manner in both the epimerization and reduction reactions. Additionally, the X-ray structure has allowed us to identify other residues that are potentially required for substrate binding and catalysis.

L10 ANSWER 16 OF 17 MEDLINE on STN DUPLICATE 8
ACCESSION NUMBER: 1999036864 MEDLINE
DOCUMENT NUMBER: PubMed ID: 9817848
TITLE: GDP-4-keto-6-deoxy-D-mannose epimerase/reductase from Escherichia coli, a key enzyme in the biosynthesis of GDP-L-fucose, displays the structural characteristics of the RED protein homology superfamily.
AUTHOR: Rizzi M; Tonetti M; Vigevani P; Sturla L; Bisso A; Flora A D; Bordo D; Bolognesi M
CORPORATE SOURCE: Dipartimento di Scienza e Tecnologia del Farmaco Universita del Piemonte Orientale "A.Avogadro" Viale Ferrucci 33-28100 Novara, Italy.
SOURCE: Structure (London, England : 1993), (1998 Nov 15) Vol. 6, No. 11, pp. 1453-65.
Journal code: 101087697. ISSN: 0969-2126.
PUB. COUNTRY: ENGLAND: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
(RESEARCH SUPPORT, NON-U.S. GOV'T)
LANGUAGE: English
FILE SEGMENT: Priority Journals
OTHER SOURCE: PDB-1BWS; PDB-1RBWSSF
ENTRY MONTH: 199901
ENTRY DATE: Entered STN: 15 Jan 1999
Last Updated on STN: 15 Jan 1999
Entered Medline: 7 Jan 1999

AB BACKGROUND: The process of guanosine 5'-diphosphate L-fucose (GDP-L-fucose) biosynthesis is conserved throughout evolution from prokaryotes to man. In animals, GDP-L-fucose is the substrate of fucosyltransferases that participate in the biosynthesis and remodeling of

glycoconjugates, including ABH blood group and Lewis-system antigens. The 'de novo' pathway of GDP-L-fucose biosynthesis from GDP-D-mannose involves a GDP-D-mannose 4,6 dehydratase (GMD) and a GDP-4-keto-6-deoxy-D-mannose epimerase/reductase (GMER). Neither of the catalytic mechanisms nor the three-dimensional structures of the two enzymes has been elucidated yet. The severe leukocyte adhesion deficiency (LAD) type II genetic syndrome is known to result from deficiencies in this de novo pathway. RESULTS: The crystal structures of apo- and holo-GMER have been determined at 2.1 A and 2.2 A resolution, respectively. Each subunit of the homodimeric (2 x 34 kDa) enzyme is composed of two domains. The N-terminal domain, a six-stranded Rossmann fold, binds NADP+; the C-terminal domain (about 100 residues) displays an alpha/beta topology. NADP+ interacts with residues Arg12 and Arg36 at the adenylic ribose phosphate; moreover, a protein loop based on the Gly-X-X-Gly-X-X-Gly motif (where X is any amino acid) stabilizes binding of the coenzyme diphosphate bridge. The nicotinamide and the connected ribose ring are located close to residues Ser107, Tyr136 and Lys140, the putative GMER active-site center. CONCLUSIONS: The GMER fold is reminiscent of that observed for UDP-galactose epimerase (UGE) from Escherichia coli. Consideration of the enzyme fold and of its main structural features allows assignment of GMER to the reductase-epimerase-dehydrogenase (RED) enzyme homology superfamily, to which short-chain dehydrogenase/reductases (SDRs) also belong. The location of the NADP+ nicotinamide ring at an interdomain cleft is compatible with substrate binding in the C-terminal domain.

L10 ANSWER 17 OF 17 MEDLINE on STN DUPLICATE 9
ACCESSION NUMBER: 1998437593 MEDLINE
DOCUMENT NUMBER: PubMed ID: 9761875
TITLE: Preliminary crystallographic investigations of recombinant GDP-4-keto-6-deoxy-D-mannose epimerase/reductase from E. coli .
AUTHOR: Tonetti M; Rizzi M; Vigevani P; Sturla L; Bisso A; De Flora A; Bolognesi M
CORPORATE SOURCE: Istituto Policattedra di Chimica Biologica, Universita' di Genova, Viale Benedetto XV, 1. I-16132 Genova, Italy.
SOURCE: Acta crystallographica. Section D, Biological crystallography, (1998 Jul 1) Vol. 54, No. Pt 4, pp. 684-6. Journal code: 9305878. ISSN: 0907-4449.
PUB. COUNTRY: Denmark
DOCUMENT TYPE: (COMPARATIVE STUDY) Journal; Article; (JOURNAL ARTICLE) (RESEARCH SUPPORT, NON-U.S. GOV'T)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199812
ENTRY DATE: Entered STN: 15 Jan 1999 Last Updated on STN: 15 Jan 1999 Entered Medline: 14 Dec 1998

AB The GDP-4-keto-6-deoxy-D-mannose epimerase/reductase (GM_ER) isolated from E. coli has been overexpressed as a GST-fusion protein and purified to homogeneity. The enzyme, an NADP+(H)-binding homodimer of 70 kDa, is responsible for the production of GDP-L-fucose. GM_ER shows significant structural homology to the human erythrocyte protein FX, which is involved in blood-group glycoconjugate

biosynthesis, displaying 3,5 epimerase/reductase activity on GDP-4-keto-6-deoxy-D-mannose. GM_ER has been crystallized in a trigonal crystalline form, containing one molecule per asymmetric unit, suitable for high-resolution crystallographic investigations.